Analysis of Diatom Blooms Using DNA Fingerprints

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LONG-TERM GOALS

My long-term goals are to understand the factors – both biological and environmental – that dictate the timing and magnitude of diatom blooms. I am particularly interested in how species behavior is coupled to environmental conditions and how the extent of genetic and physiological diversity within a population influences its future ability to bloom.

OBJECTIVES

The premise that guides my research is that phytoplankton community dynamics reflect a complicated interaction between environmental variability and the inherent genetic and physiological variation present within individual species of phytoplankton. My goal is to determine how diverse – at both a genetic and physiological level - individual species of diatoms are and how this diversity is shaped by different environments and on different time scales. Ultimately, this should allow me to determine how population diversity is coupled to future blooming capabilities.

APPROACH

Despite the critical role of diatoms in marine ecosystems, relatively little is understood about whether individual species of diatom adapt to different environmental conditions. In terrestrial and freshwater ecosystems, localized adaptation is commonly facilitated by reduced gene flow between isolated populations. In the marine environment where few obvious physical boundaries exist over vast areas, it has been harder to envision how populations of planktonic organisms with attributes adapted to particular locales could develop.

Our goal has been to determine how genetic and physiological diversity is shaped by the environment. We examine genetic diversity within diatom populations by utilizing high-throughput DNA fingerprinting techniques more traditionally used to study populations of large, multicellular organisms. Our studies focus on the unicellular centric diatom *Ditylum brightwellii* because of the importance of this diatom in coastal waters and because of the ease of identifying it in mixed populations.

We base our DNA fingerprints on highly repetitive regions of DNA known as microsatellites. These repetitive sequences are generally considered "junk" DNA with no obvious function. The beauty of these "junk" microsatellites for fingerprinting studies is that the length of any given repetitive region

can vary dramatically between individuals and so can be used to define individuals. We determine genetic diversity within *D. brightwellii* populations by first isolating individual cells into about 1 ml of media (in a 48-well plate) and then allowing the cells to divide asexually (1-2 weeks) until ~2000 cells per well are present. Each isolate can be thought of as a different individual composed of 2000 genetically identical cells. A subset of isolates is maintained in culture for physiological studies. DNA is extracted from all isolates (the DNA can be stored for later analysis) and polymerase chain reaction (PCR) is used to amplify specific microsatellites. The length of a microsatellite defines its allele size; the composite of allele sizes at different microsatellite loci defines the DNA fingerprint for an individual; the combination of individual fingerprints defines a population. We use the population-based information to determine whether genetically defined populations co-vary with different environmental conditions.

WORK COMPLETED

This year we have focused on analyzing isolates previously collected during two field studies conducted within Puget Sound, WA. The primary difference between the two studies was the time scale over which samples were collected. In the first study, *D. brightwellii* isolates were collected from different sites over the course of two and a half years. Isolates were obtained from three sites within Hood Canal sampled on a single day in November, 1997; two sites within Admiralty Inlet sampled during August, 1998 and one of the same sites within Admiralty Inlet sampled during September and October, 1998; and one site within Dabob Bay sampled in March, 2000. Five hundred and twelve single cell isolates were collected during this study with 317 surviving as unialgal isolates from which DNA could be isolated. Two hundred seventy of these isolates could be used for genotyping at three microsatellite loci (although not all isolates could be genotyped at all loci). Six isolates collected from Admiralty Inlet in August, 1998 and eight isolates collected from Hood Canal in November, 1997 were maintained in culture for physiological studies. In the second field study, we collected isolates from a single site in Dabob Bay (within Hood Canal) over the course of 11 days during a spring bloom (March/April). One thousand eight isolates were collected during this study, with 853 surviving as unialgal isolates for DNA isolation. Eight hundred twenty could be used for genotyping.

RESULTS

The work described below has been conducted by me, my graduate student, Tatiana Rynearson, and an undergraduate, Chuck Lausche. A second undergraduate, Rhonda Marohl, began working on this project with us this summer. Analysis of the data from the interannual time series has been completed and a manuscript has been submitted for publication. Analysis of the bloom data is ongoing.

In the time series study, we used genetics and physiology to determine whether distinct populations of *D. brightwellii* were present within either the Strait of Juan de Fuca or Puget Sound. For logistical simplicity, we chose one of our sampling sites to be within Admiralty Inlet, the connection between Puget Sound and the Strait of Juan de Fuca. The Inlet is a region of intense tidal mixing (current speeds can exceed 1 $m \cdot sec^{-1}$) which means that cells isolated from there must have originated from less dynamic environments within either the Strait or the Sound. Based on analysis of our Admiralty Inlet CTD data and sampling within Puget Sound itself, we determined that cells were isolated from Puget Sound waters at three different times: on 11/97, 8/98, and 3/00. We isolated cells from Strait of Juan de Fuca waters at four different times: twice on 8/98 but at two sites ¼ miles apart, on 9/98 and 10/98.

We used our DNA fingerprinting techniques to determine the extent of genetic differentiation (F_{st}) between isolates from the different water samples. If there is no genetic differentiation between populations, F_{st} is 0; as allele frequencies diverge (and populations become more genetically distinct), F_{st} increases to a theoretical maximum of 1. Three genetically distinct populations were defined: a Puget Sound population sampled over the course of more than 2 years; a Strait of Juan de Fuca population sampled in August of 1998; and a second Strait population sampled in September and October of 1998 and thus stable for at least a month (Fig. 1). The highest F_{st} values (0.245) and thus the greatest genetic differentiation occurred between samples collected from Puget Sound and those collected from the Strait of Juan de Fuca in September and October of 1998. The apparent long-term stability of a Puget Sound population and the maintenance of a Strait population over the course of two months were particularly intriguing results of this study.

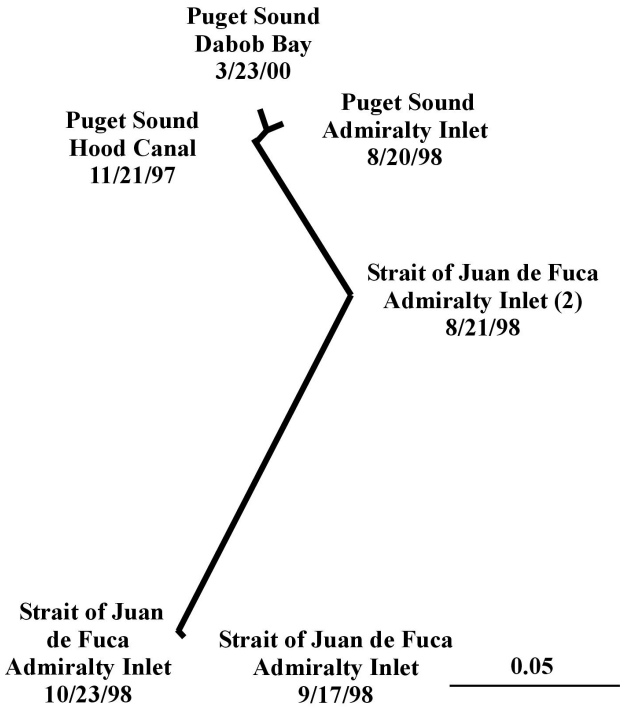

Fig. 1. Unrooted phenogram of F_{st} values showing population differentiation among samples collected from Puget Sound and the Strait of Juan de Fuca. Each tip is labeled with water mass origin, sample location and date. Scale bar indicates pair-wise tree distances. The three Puget Sound samples represent a single population. There are two Strait of Juan de Fuca populations, one defined by the two 8/21/98 samples and one defined by the 9/17/98 and 10/23/98 samples.

We also asked whether the two most genetically diverged populations were physiologically distinct as well. Maximum growth rates were measured at three different light levels. At the highest and lowest light intensities examined, the Strait of Juan de Fuca population grew significantly faster than the Puget Sound populations (Fig. 2). Only at the intermediate light intensity were the growth rates indistinguishable. These results indicate that the populations are both genetically and physiologically diverged.

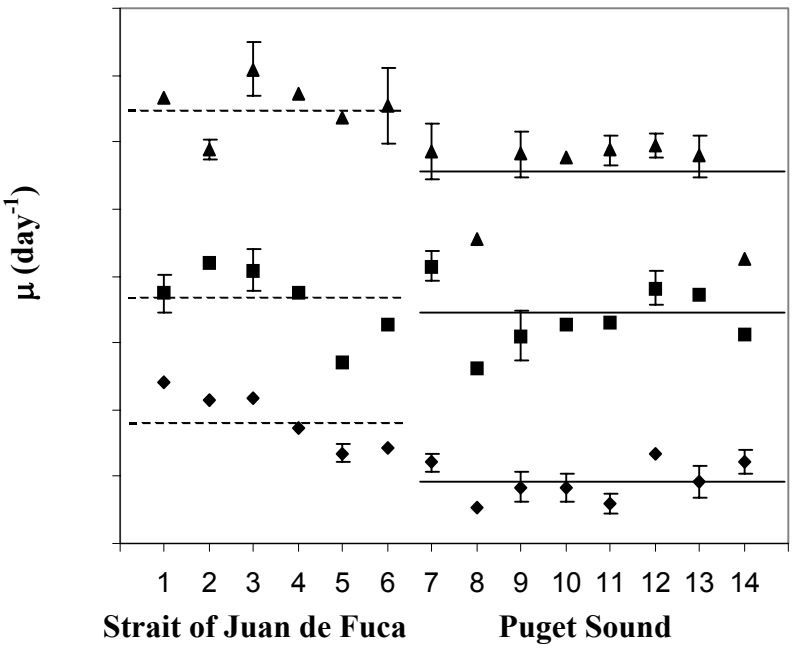

Fig. 2. Comparison of the maximum specific growth rates (day^{-1}) of isolates sampled from the Strait of Juan de Fuca (clones 1-6) and from Puget Sound (clones 7-14). The maximum growth rate of each clone was determined at 166 (triangle), 66 (square), and 33 (diamond) μmol photons m^{-2} s^{-1}. Error bars represent one standard deviation of the mean growth rate (n = 2-4) and are shown only if larger than the symbol. Horizontal lines for each light intensity illustrate the mean growth rate of all isolates from the Strait (dashed) or the Sound (solid).

How could a distinct Puget Sound population with attributes distinct from the Strait populations be maintained for over two years? Approximately $5 \cdot 10^{12}$ L of water crosses into Admiralty Inlet each flood tide bringing with each liter of water as many as 1000 Strait-derived *D. brightwellii* cells. We hypothesize that two factors are at work. First, Puget Sound-derived cells are maintained within the Sound due to a refluxing of water (~60% each tidal cycle) at the Admiralty Inlet sill. Second, strong selection appears to prevent successful reproduction of Strait-derived cells in the Sound. These results suggest that in the ocean, weak physical retention of cells in combination with differential selection can maintain uniquely adapted populations of even rapidly dividing phytoplankton. We are currently examining what factors might prevent Strait-derived cells from successfully reproducing in the Sound.

Table 1. Re-sampling of clonal cell lines during the 11 day bloom.

Times Sampled	Number Clones
1	427
2	47
3	13
4	4
5	1
6	2
7	2

One obvious prediction resulting from the interannual study was that the same resident Puget Sound population would "seed" the bloom we monitored in Dabob Bay. We have shown unequivocally that this is the case – we can predict with some certainty that we can re-sample the Puget Sound population regardless of season. We are currently analyzing this data to determine if a high level of diversity is maintained throughout the bloom, when rapid asexual division might potentially lead to the dominance of only a few fast-growing clones.

The key points that have emerged thus far are as follows. First, we have been able to show that two cells with the same three-locus fingerprint have a greater than 99.99% likelihood of being genetically identical throughout the genome and thus are members of the same clonal lineage. Using these criteria, we determined that several cell lines were re-sampled during the course of the bloom (Table 1). This

re-sampling meant that we could use "capture-recapture" statistics to estimate that the Puget Sound population was composed of ~2500 genetically distinct clones. This estimate of clonal diversity would likely increase given even more sampling of the population. Moreover, we found that certain clones were sampled at significantly higher frequency than expected suggesting that selection may have favored these lineages. On day five of the bloom, a wind-event coincided with a 51% decrease in cell number and a significant shift in abundant clonal lineages. We hypothesize that storms and other stochastic events alter environmental conditions on short time scales, preventing the eventual dominance of individual clones. This combination of high clonal diversity within a population and short-term environmental fluctuations suggests that diatom populations may be inherently resilient to environmental change, at least on a yearly time scale

IMPACT/APPLICATION

We have shown that eukaryotic microbes can adapt to local environmental conditions and that it is possible to define resident members of a community, even those members that are wholly planktonic. It appears that in the ocean, weak physical retention of cells in combination with differential selection can maintain uniquely adapted populations of rapidly dividing microbes. This new way of thinking about physical and genetic partitioning in the marine environment has ramifications for a broad range of research arenas, from management of coastal regions to theories of speciation.

TRANSITIONS

We have already begun working with Peter Franks from Scripps Institution of Oceanography to develop microsatellite-based approaches to understand dinoflagellate bloom dynamics. We have just begun discussions with European collaborators to examine Phaeocystis blooms.

PUBLICATIONS

Rynearson, T. A. and E. V. Armbrust. 2002. Genetically and physiologically distinct populations of the planktonic diatom *Ditylum brightwellii* maintained in a dynamic marine environment. Submitted.

Armbrust, E. V. and T. A. Rynearson. 2002. Diatom population dynamics: Molecular identification of genetically and physiologically distinct subpopulations of the centric diatom, *Dytilum brightwellii*. American Society of Limnology and Oceanography annual meeting.

Rynearson, T. A., and E. V. Armbrust. 2002. Who's blooming? Genetic diversity of a centric diatom during a Spring bloom. American Society of Limnology and Oceanography annual meeting.